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1034/1F811-US1

PROTEINS RELATED TO NEURONAL REGENERATION AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates generally to the treatment of neurological injury and dysfunction associated with central nervous system trauma. In particular, the invention is directed to the identification of proteins which induce neuronal regeneration.

BACKGROUND OF THE INVENTION

The peripheral nervous system (PNS) comprises highly organized groups of axon fibers or nerves external to the brain and spinal cord, such as the nerves in the limbs. In response to nerve damage, the peripheral nervous system often attempts to repair itself. While the return of lost functions is usually incomplete, generally the injured organism can adapt and function.

By contrast, damage to the central nervous system (CNS), comprising the brain and spinal cord, is generally more serious, usually causing permanent severe disability or even death.

A number of conditions are known to affect both growth and spontaneous regeneration in nerves, but the underlying mechanisms are not well understood (Gibson *et al.*, In *Compend. Contin. Educ. Pract. Vet.*, vol. 11, pp., 1989, 938–945; and Daniloff *et al.*, J. Cell Bio., 1986, 103:929–945). These conditions include the location of injury, the type of injury, the severity of injury, and the age and general health of the patient.

It has been reported that minor prior recoveries somehow prime the nerve for greater recovery in secondary lesions, for example, recovery from an earlier compression injury.

There are no previous reports of an effective treatment for injuries to neurons of the central nervous system, *i.e.*, the brain and spinal cord (*see*, M. Walker, New Engl. J. Med., 1991, 324:1885–1887.

The lack of effective treatments for nervous system injuries may be due to an insufficient understanding both of the formation of the nervous system and of its responses to injuries. Several attempts have been made to electrically stimulate injured nerves to try to cause regrowth; recovery was highly variable and inadequate (*see*, B. Sisken *et al.*, Restorative Neurology and Neuroscience, 1990, 1:303–309; see generally J. Daniloff *et al.*, "The Molecular Bases of Nerve Regeneration," in S. Malhotra (ed.), Advances in Neural Science, vol. 2, 1993). The method that is currently used most often to close gaps in severed nerves uses grafts of the patient's own sensory nerves, typically taken from the ankle; a minimal degree of recovery and permanent analgesia of the donor foot are the usual results.

Because an injured spinal cord has very limited ability to recover spontaneously, and because the consequences of spinal cord injuries can be so serious, there is a particular need for an effective treatment of spinal cord injuries. Paralytic spinal cord injuries in the United States alone occur at the rate of about 10,000 per year. Although the mortality rate is less than 10%, approximately 720 Americans per million population are permanently disabled as a result of spinal cord injuries. Most of the injured are young people in the most productive stage of life.

Following injury to neuronal cells in the central nervous system, there is often an abortive attempt by injured neural cells to generate new cellular extensions (dendrites and axons) in order to reestablish inter-neural contacts. In the central nervous system, these nerve sprouting and regeneration activities are often modest and only poorly sustained such that regeneration following stroke, trauma, spinal cord injury, etc., does not usually occur.

Thus, there is a need in the art for material and methods for treating neuronal injury.

SUMMARY OF THE INVENTION

The present invention addresses this need. Applicants have surprisingly discovered that a neuron-specific armadillo protein -- Neural Plakophilin Related Armadillo Protein (NPRAP) -- causes the development of numerous long, cellular extensions, which are similar to axonal sprouting observed during neuronal regeneration and synapse formation.

One aspect of the invention is directed to a method of stimulating growth of nerve cells, which method comprises contacting the nerve cells with an hNPRAP having nerve growth stimulating activity in an amount effective to cause nerve cell growth.

In a specific embodiment related to a method of stimulating growth of nerve cells, the method comprises contacting nerve cells with an hNPRAP stimulating agent in an amount sufficient to induce the expression of an hNPRAP and cause nerve cell growth.

A further related aspect of the invention is directed to a method of stimulating neuronal regeneration in a mammal, which method comprises administering to the mammal in need thereof an effective amount of an hNPRAP or an effective amount of an hNPRAP expression stimulating agent as set forth above.

A further aspect of the invention is related to a pharmaceutical composition comprising an hNPRAP having nerve growth stimulating activity, and a pharmaceutically acceptable carrier.

Yet another aspect of the invention is related to a pharmaceutical composition comprising an hNPRAP expression stimulating agent and a pharmaceutically acceptable carrier.

In a specific embodiment, the invention provides a pharmaceutical composition comprising an hNPRAP gene therapy vector, which vector comprises a polynucleotid encoding hNPRAP and a promoter for expressing hNPRAP, and a carrier. Naturally, such gene therarpy vectors are also part of the invention as well.

A further aspect of the invention relates to a method for identifying substances that modulate the expression of hNPRAP, which method comprises contacting cultured cells that express hNPRAP with a test substance measuring levels of hNPRAP, as compared to a control in

which the same cells that express hNPRAP are not contacted with the test substance, as an indication of modulatory activity of said test substance.

These and other aspects of the invention are disclosed more fully in the accompanying detailed description.

DETAILED DESCRIPTION

The human Neural Plakophilin Related Armadillo Protein ("hNPRAP") (also described as GT24) consensus cDNA (SEQ ID NO:3) encodes a protein (SEQ ID NO:4) of 1084 amino acid residues with a unique N-terminus, but with homology to proteins with armadillo (arm) repeat motifs at its C-terminus.

Applicants have now discovered that over-expression of hNPRAP, or functional derivatives thereof containing one or more armadillo repeats, causes the development of numerous long, dendritic processes which typically terminate upon distantly located cells. These target cells need not necessarily be expressing hNPRAP. The hNPRAP induced cellular extensions are highly similar to the axonal sprouting seen during neuronal regeneration and synapse formation.

Nucleotides 2920-2997 of the hNPRAP cDNA overlap the anonymous microsatellite locus D5S478, therefore placing the hNPRAP gene on chromosome 5p15 near the Cri-du-Chat deletion locus, a syndrome associated with congenital malformation and gross mental retardation. hNPRAP is described in detail in copending commonly assigned U.S. Application Serial Nos. 08/888,077, filed July 3, 1997 (PCT/CA97/00051), and 09/227,725, filed January 8, 1999 (PCT/CA99/00018), both of which are incorporated herein by reference.

As described in U.S. Application Serial Nos. 08/888,077 (PCT/CA97/00051) and 09/227,725 (PCT/CA99/00018), hNPRAP is known to interact with Presenilin I ("PS1") and Presenilin II ("PS2") by direct protein:protein interaction studies. The domain of the PS1 protein that interacts with hNPRAP has also been shown to interact with other proteins, such as armadillo repeat proteins p0071 and β-catenin.

On Northern blots, the hNPRAP gene is expressed as a range of transcripts of 3.9 to 5.0 kb in several regions of adult human brain, but is expressed at only very low levels in most non-neurologic tissues. Studies have shown that PS1 and hNPRAP are both expressed in the same cell types and in adjacent/contiguous subcellular compartments.

In situ hybridization studies indicate that the transcriptional pattern of PS1 and NPRAP overlap both in the brain of 4 month old mice, and in the neural tube and dorsal root ganglia of murine embryos. Both genes are expressed at high levels in dentate and hippocampal neurons, in scattered neocortical neurons, and in cerebellar Purkinje cells in adult mouse brain (Lee *et al.*, J. Neurosci., 1996, 16:7513-7525; Paffenholz and Franke, Differentiation, 1997, 61:293-304). Immunocytochemical studies show that PS1 and hNPRAP have overlapping intracellular distributions. Thus, in non-confluent transfected cell cultures, hNPRAP has a predominantly perinuclear cytoplasmic distribution contiguous with that of PS1. In contrast, in confluent cells with abundant cell:cell contacts, hNPRAP is predominantly located near the cell membrane close to inter-cellular contact zones while PS1 retains its predominantly perinuclear distribution.

The invention is directed to the use of an hNPRAP to stimulate neuronal regeneration and axon sprouting following a wide variety of insults and injuries. An "hNPRAP" is defined herein as a biologically active polypeptide that contains a sequence of hNPRAP that mediate its nerve cell growth stimulating activity, *e.g.*, the *armadillo* repeats. Thus, hNPRAP includes full-length (naturally occurring) hNPRAP, as well as biologically active analogues thereof. By "analogues" it is meant modifications such as point mutations, amino acid substitutions, additions or deletions, or other mammalian homologues, such as mouse (SEQ ID NO:5 and SEQ ID NO:6), which have similar activity to hNPRAP, the identification and selection of which are well-known to those skilled in the art. In addition to hNPRAP, the use of recombinant proteins such as p120cas and chimeric proteins having all or parts of the C-terminal armadillo-like repeat and C-terminal unique sequences of hNPRAP may also be utilized in the practice of this invention. Analogues of these proteins which replicate the effects thereof may also be utilized in the practice of this invention.

In a first embodiment, the invention provides a method of stimulating growth of nerve cells, comprising contacting nerve cells with an hNPRAP.

A second embodiment is directed to a method of stimulating growth of nerve cells, comprising contacting nerve cells with an hNPRAP stimulating agent in an amount sufficient to induce the expression of hNPRAP. Such agents may induce the expression of hNPRAP by positively binding to the hNPRAP gene to induce expression, or may alter the interaction of hNPRAP with an inhibitor of hNPRAP expression, *e.g.*, by binding to the inhibitor itself or to hNPRAP such that the inhibitor no longer modulates the expression of hNPRAP.

Alternatively, the expression of hNPRAP may be induced by the use of an appropriate viral vector system, or by the administration of recombinant proteins, biological molecules or small molecules which simulate or resemble either the *armadillo* binding domain of the presenilins or the *armadillo* repeats of hNPRAP. Another embodiment is directed to a method for identifying substances that simulate or resemble (mimic) either the *armadillo* binding domain of the presenilins or the *armadillo* repeats of hNPRAP, and which substances cause neural growth. Candidate compounds which are shown to mimic either the *armadillo* binding domain of the presenilins or the *armadillo* repeats of hNPRAP may be produced in pharmaceutically useful quantities for use in the treatment of neurological injury and dysfunction associated with central nervous system trauma. Candidate compounds include endogenous cellular components which interact with the presenilins *in vivo* and which, therefore, provide new targets for pharmaceutical and therapeutic interventions, as well as recombinant, synthetic and otherwise exogenous compounds which may have presenilin binding capacity and, therefore, may be candidates for pharmaceutical agents. Thus, in one procedure, cell lysates or tissue homogenates (*e.g.*, human brain homogenates, lymphocyte lysates) may be screened for proteins or other compounds which bind to one of the normal or mutant presenilins. Alternatively, any of a variety of exogenous compounds, both naturally occurring and/or synthetic (*e.g.*, libraries of small molecules or peptides), may be screened for presenilin binding capacity. In each of these embodiments, an assay is conducted to detect binding between a presenilin component containing at least the interacting domain of a presenilin protein described herein and some other moiety.

As described in U.S. Application Serial No. 09/227,725, the presenilin domain that interacts with PS-interacting proteins, such as armadillo repeat proteins hNPRAP, p0071 and β-catenin, has been identified as including or being contained in the sequence of amino acid residues from about 260 to about 409 of PS1 or corresponding residues from about 260 to about 390 in PS2. More preferably, the interacting domain contains or is contained in amino acid residues from about 372 to about 399 of PS1 or corresponding residues from about 350 to about 380 in PS2. The amino acid sequences of wild-type human PS1 and PS2 are shown in SEQ ID NO:1 and SEQ ID NO:2, respectively.

Binding may be detected by indirect functional measures reflecting the functional consequences of the interaction (*e.g.*, changes in intracellular Ca^{2+}, Na^{+}, K^{+}, or GTP/GDP ratio, changes in apoptosis or microtubule associated protein phosphorylation, changes in Aβ peptide production or changes in the expression of other downstream genes which can be monitored by differential display, 2D gel electrophoresis, differential hybridization, or SAGE methods) or by direct measures such as immunoprecipitation, the Biomolecular Interaction Assay (BIAcore) or alteration of protein gel electrophoresis. The preferred methods involve variations on the following techniques: (1) direct extraction by affinity chromatography; (2) co-isolation of presenilin components and bound proteins or other compounds by immunoprecipitation; (3) BIAcore analysis; and (4) the yeast two-hybrid systems. Other procedures include methods which detect abnormal processing of PS1, PS2, APP, or proteins reacting with PS1, PS2, or APP (*e.g.*, abnormal phosphorylation, glycosylation, glycation amidation or proteolytic cleavage) alterations in presenilin transcription, translation, and post-translational modification; alterations in the intracellular and extracellular trafficking of presenilin gene products; or abnormal intracellular localization of the presenilins.

The proteins or other compounds identified by these methods may then be assayed for their ability to promote sprouting in axons of neuronal cultures or dendrite formation in non-neurological cells using morphometric analyses which are well-known to those skilled in the art of neuronal regeneration. Alternatively, assays for regeneration following sectioning of the optic

nerve, spinal cord, etc. in animals may be performed. Such assays are well-known to those in the field of neuronal regeneration.

The proteins or other compounds identified by these methods may be purified and characterized by any of the standard methods known in the art. Proteins may, for example, be purified and separated using electrophoretic (*e.g.*, SDS-PAGE, 2D PAGE) or chromatographic (*e.g.*, HPLC) techniques and may then be microsequenced. For proteins with a blocked N-terminus, cleavage (*e.g.*, by CNBr and/or trypsin) of the particular binding protein is used to release peptide fragments. Further purification/characterization by HPLC and microsequencing and/or mass spectrometry by conventional methods provides internal sequence data on such blocked proteins. For non-protein compounds, standard organic chemical analysis techniques (*e.g.*, IR, NMR and mass spectrometry; functional group analysis; X-ray crystallography) may be employed to determine their structure and identity.

These hNPRAPs, and compounds which activate hNPRAP, may be employed in combination with a suitable pharmaceutical, physiologically acceptable carrier. Administration of hNPRAP of this invention can be through the administration of hNPRAP peptides agonists or antagonists synthesized from recombinant constructs of hNPRAP DNA or from peptide chemical synthesis (Woo, *et al.*, Protein Engineering, 1989, 3:29-37) or in the form of gene therapy (Goldspiel *et al.*, Clin. Pharm., 1993, 12:488; Wu and Wu, Biotherapy, 1991, 3:87; Mulligan, Science, 1993, 260:926; Morgan and Anderson, Ann. Rev. Biochem., 1993, 62:191; and, May TIBTECH, 1993, 11:155).

Generally, hNPRPA and/or activating agent(s) are administered as pharmaceutical compositions comprising an effective amount of hNPRAP and/or activating agent(s) in a pharmaceutical carrier. These reagents can be combined for therapeutic use with additional active or inert ingredients, *e.g.*, in conventional pharmaceutically acceptable carriers or diluents, *e.g.*, immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivering the compositions of the invention to a patient.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Animal testing of effective doses for treatment of particular injuries will provide further predicative indication of human dosage. Various considerations are described, *e.g.*, in Gilman *et al.* (eds.) (1990) Goodman and Gilman's; The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, PA. Methods for administration are discussed therein and below, *e.g.*, for intravenous, intraperitoneal, or intramuscular administration, transfermal diffusion, and others. Pharmaceutically acceptable carriers include water, saline, buffers and other compounds described, *e.g.*, in the Merck Index, Merck & Co., Rahway, New Jersey, and in Remington, *supra*. Slow release formulations, or a slow release apparatus, may be used for continuous administration.

Dosage ranges for hNPRAP and/or activating agent(s) would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstance is reached. Determination of the proper dosage and administration regime for a particular situation is within the skill of the art.

Polypeptides and other compounds of the present invention which activate or inhibit hNPRAP may be employed alone or in conjunction with other compounds, such as therapeutic compounds. Once identified by the methods described above, the candidate compounds may then be produced in quantities sufficient for pharmaceutical administration or testing (*e.g.*, mg or mg or greater quantities), and formulated in a pharmaceutically acceptable carrier (*see*, *e.g.*, *Remington's*, *supra*).

Pharmaceutically acceptable carriers that may be used in these pharmaceutical compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The compositions of the present invention may be administered orally, parenterally, by spray inhalation, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously.

Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers

include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with our without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

The methods and compositions of this invention may be used to treat nerve damage caused by a wide variety of diseases or physical traumas. These include, but are not limited to, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis, stroke and ischemia associated with stroke, neural paropathy, other neural degenerative diseases, motor neuron diseases, sciatic crush, peripheral neuropathy, particularly neuropathy associated with diabetes, spinal cord injuries and facial nerve crush.

The hNPRAP polynucleotides, polypeptides, agonists and antagonists that are polypeptides may be employed in accordance with the present invention by expression of such polypeptides in treatment modalities often referred to as "gene therapy". Thus, for example, cells from a patient may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*. The engineered cells can then be provided to a patient to be treated with the polypeptide. In this embodiment, cells may be engineered *ex vivo*, for example, by the use of a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention. Such methods are well-known in the art and their use in the present invention will be apparent from the teachings herein.

Similarly, cells may be engineered in vivo for expression of a polypeptide *in vivo* by procedures known in the art. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral or viral vector, as discussed above. The retroviral expression construct may then be isolated. A packaging cell is transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention, such that the packaging cell now produces infectious viral particles containing the gene of interest. These viral particles may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention should be apparent to those skilled in the art from the teachings of the present invention.

Retroviruses or viruses from which the plasmid vectors hereinabove-mentioned may be derived include, but are not limited to, SimiForest Virus, Lenti-virus, Moloney Murine Leukemia Virus, Spleen Necrosis Virus, Rous Sarcoma Virus, Harvey Sarcoma Virus, Avian Leukosis Virus, Gibbon Ape Leukemia Virus, Human Immunodeficiency Virus, Adenovirus, Myeloproliferative Sarcoma Virus, and Mammary Tumor Virus. In a preferred embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

Such vectors will include one or more promoters for expressing the polypeptide. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller *et al.*, Biotechniques, 1989, 7:980-990. Cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, RNA polymerase III, and β-actin promoters, can also be used. Additional viral promoters which may be employed include, but are not limited to, adenovirus promoters such as the adenoviral major late promoter, thymidine kinase (TK) promoters such as the Herpes Simplex thymidine kinase promoters; the respiratory syncytial virus (RSV) promoters; and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention may be placed under the control of an inducible promoter. Suitable inducible promoters which may be

employed include, but are not limited to, the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters; and human growth hormone promoters. The promoter may also be the native promoter which controls the gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, .PSI.-2, .omega.-AM, PA12, T19-14X, VT-19-17-H2, .omega.CRE, .omega.CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, A., Human Gene Therapy, 1990, 1:5-14. The vector may be transduced into the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host. The producer cell line will generate infectious retroviral vector particles, which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles may then be employed to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

* * *

Although the invention herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the principles and applications of the present invention. It is therefore to be understood that numerous modifications may be made to the illustrative embodiments and that other arrangements may be devised without departing from the spirit and scope of the present invention as defined by the appended claims.

Each patent, patent application, publication, and procedure disclosed in this application is specifically incorporated by reference in its entirety.